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SHORT COMMUNICATION

Angiotensin II: a hormone that affects lipid metabolism in adipose tissue

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Background: Alterations in adipose tissue lipolysis may contribute to the pathophysiology of obesity and insulin resistance. We examined the effects of angiotensin II (Ang II) on abdominal subcutaneous adipose tissue lipolysis in humans.

Methods and results: First, adipocytes obtained from nine normal weight and seven obese subjects were stimulated with Ang II (10^{-14} – 10^{-6} M). Glycerol concentration in the medium, used as an indicator of adipocyte lipolysis, was significantly reduced (~20%) after Ang II stimulation in adipocytes from normal weight ($P=0.04$) and obese subjects ($P<0.001$). Based on these observations, adipocytes of seven additional obese subjects were stimulated with lower doses of Ang II (10^{-17} – 10^{-6} M) in the presence and absence of Ang II type 1 (AT₁) receptor blockade. Lipolysis was dose dependently inhibited by ~20 to 25% after Ang II stimulation ($P=0.001$). AT₁ receptor blockade completely abolished the Ang II-induced effects ($P=0.35$).

Conclusion: Ang II directly inhibits abdominal subcutaneous adipocyte lipolysis in normal weight and obese subjects via the AT₁ receptor.

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Keywords: angiotensin II; Ang II type 1 receptor; lipolysis; adipose tissue

Introduction

Abdominal obesity is strongly associated with insulin resistance. Part of this association may be explained by adipose tissue products that exert autocrine, paracrine and/or endocrine effects that may affect metabolism.^{1–3}

A functional renin–angiotensin system (RAS) is present in human adipose tissue,^{4–6} and local generation of angiotensin II (Ang II), the effector molecule of the RAS, by human (pre)adipocytes has been demonstrated.⁷ The adipose tissue RAS may be involved in obesity-related disorders, such as insulin resistance,^{8,9} possibly through an effect on adipocyte differentiation.^{10,11} Furthermore, direct lipogenic effects of Ang II in human adipocytes have been shown,¹² and recent data from our laboratory suggest that Ang II may inhibit lipolysis *in vivo* in human adipose tissue and skeletal muscle.¹³ However, no conclusions about the magnitude of the observed antilipolytic effects of Ang II could be drawn from these findings, as the Ang II-induced decrease in tissue

blood flow may have masked direct effects on lipolysis to some extent. The objective of the present study was to investigate for the first time the direct effects of Ang II on abdominal subcutaneous adipose tissue lipolysis *in vitro* in normal weight and obese subjects.

Materials and methods

Nine normal weight and seven obese male subjects participated in the first study. Seven additional obese subjects participated in a second experiment. Characteristics of the subjects are summarized in Table 1. The Medical-Ethical Committee of Maastricht University approved the study protocol and all subjects gave written informed consent before participating in the study.

Protocol

Abdominal subcutaneous adipose tissue (~2 g) was obtained by needle aspiration under local anesthesia after an overnight fast. Adipocytes were isolated from the subcutaneous fat tissue specimen as described previously.¹⁴ Firstly, diluted suspensions of adipocytes (~5000–10 000 cells/incubation) from normal weight and obese subjects were incubated with

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Table 1 Subjects' characteristics

	Normal weight	Obese (study 1)	Obese (study 2)
Age (years)	54 ± 2	51 ± 2	52 ± 4
Weight (kg)	76.5 ± 1.9	112.8 ± 6.4*	109.1 ± 6.3 [#]
Height (m)	1.78 ± 0.03	1.78 ± 0.02	1.81 ± 0.02
BMI (kg/m ²)	24.3 ± 0.4	35.5 ± 1.8*	33.3 ± 1.9 [#]
Body fat (%)	22.9 ± 1.3	34.6 ± 2.7*	34.4 ± 3.1 [#]

Abbreviation: BMI, body mass index. Values are means ± s.e.m. * $P = 0.001$ vs normal weight by unpaired *t*-test; [#] $P < 0.005$ vs normal weight by unpaired *t*-test.

Ang II (10^{-14} – 10^{-6} M; Sigma-Aldrich, Zwijndrecht, The Netherlands) for 2 h at 37°C in Krebs–Ringer phosphate buffer. Thereafter, incubation medium was immediately frozen in liquid nitrogen and stored at -80°C until analysis. Glycerol concentration in the medium, determined using a sensitive automated bioluminescence method,¹⁵ was used as an indicator of lipolysis.

Adipocytes of an additional group of obese subjects were stimulated with lower Ang II concentrations (10^{-17} – 10^{-6} M) in the presence and absence of AT₁ receptor blockade (10^{-4} M losartan; MSD, Haarlem, The Netherlands). Furthermore, adipocytes were incubated with *N*⁶-(2-phenylisopropyl)adenosine (PIA; 100 nM) (Sigma-Aldrich) as a control point for maximal inhibition of lipolysis in the incubation system.

Statistical analysis. Data are mean ± standard error of the mean (s.e.m.). Lipolytic rates are presented as relative changes from baseline because of interindividual variation in baseline glycerol concentrations. Effects of Ang II were compared by two-way repeated measures analysis of variance (ANOVA), using dose as within-subject factor and group or treatment as between-subject factor. One-way repeated measures ANOVA was performed to identify dose effects. $P < 0.05$ was considered to be statistically significant, using SPSS 10.1 (Chicago, IL, USA).

Results and discussion

Basal lipolytic rates were not significantly different between normal weight and obese subjects (4.8 ± 1.1 vs $11.4 \pm 5.1 \mu\text{mol} \times 10^7 \text{ cells}^{-1} \times 2 \text{ h incubation}^{-1}$, respectively, $P = 0.24$). Ang II significantly inhibited adipocyte lipolysis in normal weight ($P = 0.04$) and obese subjects ($P < 0.001$) (Figure 1a), with no differences between groups ($P = 0.40$). In the second experiment, basal lipolytic rate was $5.0 \pm 1.0 \mu\text{mol}/10^7 \text{ cells}/2 \text{ h incubation}$. Ang II dose dependently inhibited lipolysis by ~20–25% ($P = 0.001$), and this effect was completely abolished by AT₁ receptor blockade ($P = 0.35$) (Figure 1b).

These findings demonstrate that Ang II inhibits lipolysis through the AT₁ receptor in abdominal subcutaneous adipocytes in normal weight and obese subjects. PIA, used as a control point for maximal inhibition of lipolysis in the incubation system,¹⁶ reduced the lipolytic rate by ~40%

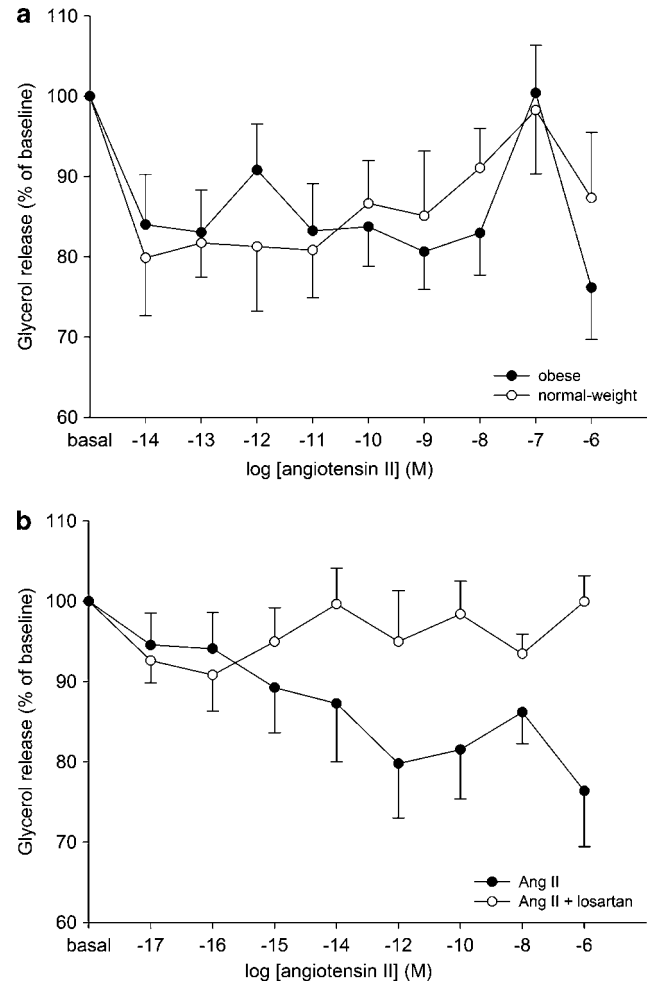


Figure 1 (a) Effects of Ang II stimulation on glycerol release from abdominal subcutaneous adipocytes from nine normal weight and seven obese subjects. Ang II reduced glycerol release both in normal weight ($P = 0.04$ by one-way repeated-measures ANOVA) and obese subjects ($P < 0.001$ by one-way repeated-measures ANOVA), with no differences between groups ($P = 0.40$ by two-way repeated-measures ANOVA). Values are means ± s.e.m. (b) Effects of Ang II stimulation on glycerol release from abdominal subcutaneous adipocytes from seven obese subjects in the presence and absence of AT₁ receptor blockade (10^{-4} M). Glycerol release was dose dependently reduced during stimulation with Ang II ($P = 0.001$ by one-way repeated-measures ANOVA). Ang II had no significant effects on lipolysis in the presence of AT₁ receptor blockade ($P = 0.35$ by one-way repeated-measures ANOVA). Values are means ± s.e.m.

($P < 0.01$) (data not shown). Therefore, the observed ~20–25% reduction of glycerol release after Ang II stimulation reflects a substantial inhibition of lipolysis. The normal circulating Ang II concentration is 10 pM.¹⁷ It has previously been shown that Ang II concentrations are ~2–3-fold higher in the incubation medium of (pre)adipocytes than in the circulation.^{4,7} Because stimulation of adipocytes with physiological concentrations of Ang II evoked near-maximal inhibition of lipolysis in the present experiments, Ang II may not play an important role in the regulation of adipocyte lipolysis. Instead, Ang II may exert a tonic suppression of adipocyte lipolysis.

The present findings are in line with previous observations suggesting that Ang II inhibits adipose tissue lipolysis in humans.¹³ In contrast, no effect of Ang II on insulin-induced suppression of lipolysis was observed in obese women.¹⁸ In addition to methodological differences compared with the present study, insulin may have masked a less pronounced antilipolytic effect of Ang II. In a rat model for increased RAS activity in cachectic patients with advanced heart failure, lipolysis was increased in some but not all fat depots.¹⁹ Because expression of (anti)lipolytic receptors differs between species,¹⁹ and the high Ang II concentrations activated the sympathetic nervous system, it is difficult to extrapolate these findings to Ang II effects on adipocyte lipolysis in humans.

It has been shown that Ang II exerts lipogenic effects in human adipocytes¹² and inhibits adipocyte differentiation.¹¹ Although Ang II effects in adipose tissue may not be involved in the expansion of fat mass, they may contribute to a reduced buffering capacity for lipid storage in adipose tissue in the long term, leading to an excessive influx of lipids to other tissues.¹⁰

In conclusion, Ang II inhibits lipolysis via the AT₁ receptor in abdominal subcutaneous adipocytes from normal weight and obese subjects. The present findings support the concept that the RAS in adipose tissue participates directly in fat metabolism and may contribute to the metabolic disturbances seen in obesity. This study was not designed to examine underlying signalling pathways, but focused on lipolysis as cellular end point. Identification of the underlying mechanisms for the antilipolytic effect of Ang II in human adipocytes may provide better insight into possible interactions between (anti)lipolytic pathways.

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